Differences of Phenotype and Gene Frequency by C System in Canine Species

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ABSTRACT. The canine C blood group was determined by an agglutination reaction with a plant agglutinin extracted from the seeds of *Clerodendron trichotomum* (CTL). A positive agglutination reaction was classified as type C and a negative reaction as type c. In this study, the C phenotype and the gene frequency of C were examined in 377 dogs (224 purebred dogs of 29 breeds and 153 mongrel dogs) that were bred in Utsunomiya-city, Tochigi Prefecture. Phenotype C was less frequent than phenotype c in every purebred dog. A variation in C gene frequency was observed among the purebred dogs. The highest frequency of C allele was in the Yorkshire Terrier (0.202), followed by Beagle, Shiba (0.091), Maltese (0.085), Shi Tzu (0.051) and Shetland Sheepdog (0.036). The C blood group system as a genetic marker may provide useful information for breed identification, genetic studies between breeds, and pedigree certification.—**KEY WORDS:** C system, gene frequency, plant agglutinin.

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With advances in veterinary medicine, many blood tests have been performed for the diagnosis and treatment of canine diseases. However, blood groups of pet dogs have not been examined much. Usually, a blood group compatibility test is performed only before an emergency blood transfusion. In contrast to humans, dogs don't possess the strong natural antibodies, so that the danger of a serious reaction from a single transfusion is small. However, with the extension of the average lifespan of dogs, the possibility of repeated blood transfusion is more likely, making blood group determination in domestic dogs extremely important [1]. The popularization of blood grouping in domestic dogs is hindered by the facts that the antibodies for blood group determination are not freely available, and this examination in laboratories is usually expensive and time-consuming.

We performed canine blood grouping using a plant agglutinin which is relatively easily accessible. Although this blood group system has already been described by Yoshida and Ikemoto *et al.*, only mongrels and Beagles have been examined [2, 9]. The inheritance was controlled by a dominant allele at an autosomal locus. We have reported the possibility that C antigen was a diagnostic marker of canine cancer. In this study, we performed blood grouping in 224 purebreds (29 breeds) and 153 mongrels (total 377) collected in Tochigi Prefecture.

The *Clerodendron trichotomum* seeds were collected in Nasu area of Tochigi Prefecture, Japan. One gram of the selected dried seeds was homogenized in 10 ml of physiological saline and stored at 4°C overnight. After centrifugation at 1,000 ×g for 10 min, the supernatant was sterilized by filtration with a Millex-GV filter (Millipore Corporation; Japan). The *Clerodendron trichotomum* seed extract diluted 1 in 256 in physiological saline was used as the plant agglutinin (CT).

Canine blood was collected by venipuncture from the vena radialis. The red blood cells were separated, washed three times with physiological saline and a 3% red blood cell suspension was prepared in physiological saline. Blood grouping was performed within 2 days of blood collection.

As simple a blood grouping method as possible was adopted. Two drops of CT (about 40 µl) and 2 drops of

3% red blood cells suspension were mixed in a small test tube, allowed to stand at room temperature for 15 min and centrifuged at 1,000 ×g for 1 min. The agglutination reaction was determined visually. A positive agglutination reaction was classified as type C, and a negative reaction was classified as type c.

The gene frequencies and X^2 test were calculated as described by Yasuda [8] and Kimura *et al.* [3, 4].

The frequency of phenotype C was 17.4% in Beagles, 16.2% in Maltese, 7.0% in Shetland Sheepdogs, 17.2% in Shiba, 10.0% in Shi Tzu, 36.4% in Yorkshire Terriers and 19.0% in mongrels (Table 1).

The frequencies of phenotype C in canine breeds with a smaller sample size (C type numbers / Sample size) were Cocker Spaniel (1/1), Collie (1/2), Dachshund (1/4), Papion (1/3), Poodle (2/8), Pug (1/3), Schnauzer (1/1), Shepherd (1/4), Welsh Corgi (1/2). In other breeds with small sample size, the phenotype c frequencies was Afghan Hound (0/1), Akita (0/3), Alaskan Malamute (0/1), Betrinton Terrier (0/1), Chin (0/1), Chow Chow (0/2), Labrador Retriever (0/5), Miniature Pincher (0/1), Pointer (0/3), Pomeranian (0/9), Setter (0/3), Siberian Husky (0/1), Spitz (0/1), and West Highland White Terrier (0/1). We will examine a large sample in the future.

The gene frequency of C was 0.091 in Beagles, 0.085 in Maltese, 0.036 in Shetland Sheepdogs, 0.091 in Shiba, 0.051 in Shi Tzu, 0.202 in Yorkshire Terriers and 0.100 in mongrels (Table 1).

Table 1. Distribution of phenotype and gene frequencies in C blood system of various canine breeds

Breeds	Numbers		Percents of phenotype	Gene frequencies
	Tested	Observed Type C		
Shetland Sheepdog	43	3	7.0	0.036
Maltese	37	6	16.2	0.085
Shiba	29	5	17.2	0.091
Beagle	23	4	17.4	0.091
Shi Tzu	20	2	10.0	0.051
Yorkshire Terrier	11	4	36.4	0.202
Mongrel	153	29	19.0	0.100

Table 2. χ^2 test of gene frequencies in C blood system of various canine breeds

	Shetland Sheepdog	Maltese	Shiba	Beagle	Shi Tzu	Yorkshire Terrier	Mongrel
Shetland Sheepdog							
Maltese	$\chi^2=1.765$ $0.20>P>0.10$						
Shiba	$\chi^2=1.580$ $0.30>P>0.20$	$\chi^2=0.013$ $0.95>P>0.90$					
Beagle	$\chi^2=1.798$ $0.20>P>0.10$	$\chi^2=0.015$ $0.95>P>0.90$	$\chi^2=0.0002$ $0.99>P>0.98$				
Shi Tzu	$\chi^2=0.150$ $0.70>P>0.50$	$\chi^2=0.429$ $0.70>P>0.50$	$\chi^2=0.524$ $0.50>P>0.30$	$\chi^2=0.505$ $0.50>P>0.30$			
Yorkshire Terrier	$\chi^2=7.158$ $0.01>P$	$\chi^2=2.394$ $0.20>P>0.10$	$\chi^2=5.655$ $0.02>P>0.01$	$\chi^2=1.678$ $0.20>P>0.10$	$\chi^2=3.539$ $0.10>P>0.05$		
Mongrel	$\chi^2=2.309$ $0.20>P>0.10$	$\chi^2=0.153$ $0.70>P>0.50$	$\chi^2=0.048$ $0.90>P>0.80$	$\chi^2=0.033$ $0.90>P>0.80$	$\chi^2=0.973$ $0.50>P>0.30$	$\chi^2=2.274$ $0.20>P>0.10$	

χ^2 test were carried out with 6 breeds and the mongrel group, all of which contained more than 10 dogs, including the Shetland Sheepdog, Maltese, Shiba, Beagle, Shi Tzu, Yorkshire Terrier. There were significant differences between Shetland Sheepdog and Yorkshire Terrier ($\chi^2 = 7.158$, d.f. = 1, $0.01>P$), and Shiba and Yorkshire Terrier ($\chi^2 = 5.655$, d.f. = 1, $0.02>P>0.01$) (Table 2).

In this study, phenotype and gene frequencies of the C blood group could be determined only in Beagle, Maltese, Shetland Sheepdog, Shiba, Shi Tzu and Yorkshire Terrier; the other breeds containing 1 to 9 dogs could not be analyzed statistically. Nevertheless, this is the first study of blood grouping using a plant agglutinin with a number of breeds.

The results of this study showed that the C system may be used as a marker in the investigations of the origin and mixed parentage of dogs, and in pedigree certification. In addition, we previously reported the possibility of CT as a diagnostic marker for canine cancer [6, 7]. CT is an interesting plant agglutinin with importance in the veterinary clinical field not limited to blood grouping.

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